

## Chromosome 3, 84 cM

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**BACKGROUND:** Dr. Y. Ji, University of Florida, indicated that there was an introgression associated with Ty4 gene on chromosome 3 near 76-85 cM. Originally, Ty4 was thought to be on chromosome 6 between 40-80 cM. Our UW-team scanned this region at 5-cM intervals and did not find any evidence for an introgression in Gc171, which gives the SCAR marker for Ty4. With this new information and the information from Dr. Ji, our group starting scanning chr. 3 from 66 - 85 cM to check for an introgression. Begomovirus resistant inbred used was G70, which was a selection from Gc171 by a susceptible hybrid. The susceptible germplasm was HUJ-VF (lab code, W168, an inbred from Hebrew University of Jerusalem, F. Vidavski and H. Czosnek) and M82.

### Primers

P3-84 F2: GCAGCAGCATCCCTAGTAGGCTTTGC

P3-84 R2: GGTAGTAGCATCTGGAATTGATGAAC

Annealing temperature: 55 C

### Partial Sequence of G70 (resistant inbred), p3-84

```
1      GAACATTGTG AGTTTGGCTT CATCTCCATT GTCTGGAAAA TAATCTTTGA GAGCATTGTA
61     CCCCTAAAAC AAGCAACAAA AAAGAAACTA TAAACATCAA AAGTGTGAG CTACACTTAA
121    TTTCTGTCTT CACCTTGTC TCCCTTTATA GGAGGCAAGT TCACTTCTGT TCTTGACCAG
181    TTATCTGCTA AGAAAGTCCT CCGTACTCCA GAGGGTTGAA AGACTCAAGT CTTTGACTAA
241    AAAAAAGTCC AAGAAAAGAAC TTAAAGAGTG ATAAACATTC TCTCACATCT ACAGCCTTAG
301    TGGGGGAGAA GGGGAGAGAG AGAGCAAAAA AGAGATGGCA AGCTTTACCA GATAGCGTGA
361    CACCTGTCCG AACTTCAACA AGTACTTCAA TGTGTTTTGA ACTAAAAGTC CAGCAACAAC
421    TCCCTATCAA AGAGAGTTC AATGTTAATT TACCAAATCA AATAATGAAT TTGTAATACA
481    GCTTCAATCA AATCATAAAC AAGATTCTAG TATTTTTGCT AGAAGAGAAG TTCATATTAT
541    TCTTCAAAAAG AAAGTGGGAG CATAAACATG AGAAAAGTAA CCAAATTAGC AGCAAATGCG
601    GCTATAAAGA GAGAGAATAA TTTTGAAGAT GAGAAGAGCC AACTTCAAC CACAATTTTC
661    AGTGTTCCTT TTTACAATTA CAATTGCTTA TCATTTGAAA GTCATTCACA TGGTGATCTT
721    CAGGCAGAGA ACTTAGAATN TGTCATGCAT AAAATTTATA AGGAGAAAGG GAA
```

### Partial Sequence of M82 (Susceptible), p3-84

None

### Partial Sequence of W168 (Susceptible), p3-84

```
1      ASSEMBLYFSE QUENCESINT GRUPGGACCA TGGACACTAT ACCTGTCGTT CCAAACAAGC
61     AGCATTTGAA CATTGTGAGT TTGGCTTCAT CTCCATTGTT GGAAAATAAT CTTTGAGAGC
121    ATTGTACCCC TAAAACAAGC AACAAAAAAG AAACATATAA CATCAAAAGT GTTGAGCTAC
181    ACTTAATTTT TGTCTTCACC TTGTCTCCTT TTTATAGGAG GCAAGTTCAC TTCTGTTCCT
241    GACCAGTTAT CTGCTAAGAA AGTCCTCCGT ACTCCAGAGG GTTGAAAGAC TCAAGTCTTT
301    GACTAAAAAA AAGTCCAAGA AAGAACTTAA AGAGTGATAA ACATTCTCTC ACATCTACAG
361    CCTTAGTGGG GGAGAAGGGG AGAGAGAGAG CAAAAAAGAG ATGGCAAGCT TTACCAGATA
421    GCGTGACACC TGTCCGAACT TCAACAAGTA CTTCAATGTG TTTTGAAC TAAGTCCAGC
481    AACAACTCCC TATCAAAGAG AGTTCAAATG TTAATTTACC AAATCAAATA ATGAATTTGT
```

541 AATACAGCTT CAATCAAATC ATAAACAAGA TTCTAGTATT TTTGCTAGAA GAGAAGTTCA  
 601 TATTATTCTT CAAAAGAAAG TGGGAGCATA AACATGAGAA AAGTAACCAA ATTAGCAGCA  
 661 AATGCGGCTA TAAAGAGAGA GAATAATTTT GAAGATGAGA AGAGCCAAAC TTCAACCACA  
 721 ATTTTCAGTG TTTCTTTTCA CAATTACAAT TGCTTATCAT TTGAAAGTCA TTCACATGGT  
 781 GATCTTCAGG CAGAGAACTT AGAATATGTC ATGCATAAAA TTTATAAGGA GAAAGGGAAC  
 841 A

**Comparison of W168 (top sequence) with G70 (bottom sequence)**

68 GAACATTGTGAGTTTGGCTTCATCTCCATTGTTGGAAAATAATCTTTGAGAGCATTGTA  
 |||||  
 1 GAACATTGTGAGTTTGGCTTCATCTCCATTGTTGGAAAATAATCTTTGAGAGCATTGTA  
  
 127 CCCCTAAAACAAGCAACAAAAAGAACTATAAACATCAAAGTGTTGAGCTACACTTAA  
 |||||  
 61 CCCCTAAAACAAGCAACAAAAAGAACTATAAACATCAAAGTGTTGAGCTACACTTAA  
  
 187 TTTCTGTCTTCACCTTGCTCCTTTTTATAGGAGGCAAGTTCACCTTCTGTTCTTGACCAG  
 |||||  
 121 TTTCTGTCTTCACCTTGCTCCTTTTTATAGGAGGCAAGTTCACCTTCTGTTCTTGACCAG  
  
 247 TTATCTGCTAAGAAAGTCTCCGTACTCCAGAGGGTTGAAAGACTCAAGTCTTTGACTAA  
 |||||  
 181 TTATCTGCTAAGAAAGTCTCCGTACTCCAGAGGGTTGAAAGACTCAAGTCTTTGACTAA  
  
 307 AAAAAAGTCCAAGAAAGAAGTAAAGAGTGATAAACATTCTCTCACATCTACAGCCTTAG  
 |||||  
 241 AAAAAAGTCCAAGAAAGAAGTAAAGAGTGATAAACATTCTCTCACATCTACAGCCTTAG  
  
 367 TGGGGGAGAAGGGGAGAGAGAGAGCAAAAAAGAGATGGCAAGCTTTACCAGATAGCGTGA  
 |||||  
 301 TGGGGGAGAAGGGGAGAGAGAGAGCAAAAAAGAGATGGCAAGCTTTACCAGATAGCGTGA  
  
 427 CACCTGTCCGAAGTTCACAAGTACTTCAATGTGTTTTGAACTAAAAGTCCAGCAACAAC  
 |||||  
 361 CACCTGTCCGAAGTTCACAAGTACTTCAATGTGTTTTGAACTAAAAGTCCAGCAACAAC  
  
 487 TCCCTATCAAAGAGAGTTCAAATGTTAATTTACCAAATCAAATAATGAATTTGTAATACA  
 |||||  
 421 TCCCTATCAAAGAGAGTTCAAATGTTAATTTACCAAATCAAATAATGAATTTGTAATACA  
  
 547 GCTTCAATCAAATCATAACAAGATTCTAGTATTTTGGCTAGAAGAGAAGTTCATATTAT  
 |||||  
 481 GCTTCAATCAAATCATAACAAGATTCTAGTATTTTGGCTAGAAGAGAAGTTCATATTAT  
  
 607 TCTTCAAAGAAAGTGGGAGCATAAACATGAGAAAAGTAACCAAATTAGCAGCAAATGCC  
 |||||  
 541 TCTTCAAAGAAAGTGGGAGCATAAACATGAGAAAAGTAACCAAATTAGCAGCAAATGCC  
  
 667 GCTATAAGAGAGAGAATAATTTTGAAGATGAGAAGAGCCAACTTCAACCACAATTTTC  
 |||||  
 601 GCTATAAGAGAGAGAATAATTTTGAAGATGAGAAGAGCCAACTTCAACCACAATTTTC  
  
 727 AGTGTTCCTTTTACAATTACAATTGCTTATCATTTGAAAGTCATTACATGGTGATCTT  
 |||||  
 661 AGTGTTCCTTTTACAATTACAATTGCTTATCATTTGAAAGTCATTACATGGTGATCTT  
  
 787 CAGGCAGAGAACTTAGAATATGTCATGCATAAAAATTTATAAGGAGAAAGGGAACA  
 |||||  
 721 CAGGCAGAGAACTTAGAATATGTCATGCATAAAAATTTATAAGGAGAAAGGGAACA

## Blast Search

There were no resulting matches with greater than 90% accuracy on NCBI database. There were several matches over 90% from the SOL genomics website, and this data is as follows:

[SGN-M6508](#) C2\_At1g05350 [cosii\_markers]

Length = 945

Score = 153 bits (77), Expect = 4e-37  
Identities = 77/77 (100%), Frame = +1 / +1

```
Query: 348 ccagatagcgtgacacctgtccgaacttcaacaagtacttcaatgtggtttgaactaaaa 407
      |||
Sbjct: 694 ccagatagcgtgacacctgtccgaacttcaacaagtacttcaatgtggtttgaactaaaa 753
```

```
Query: 408 gtccagcaacaactccc 424
      |||
Sbjct: 754 gtccagcaacaactccc 770
```

Score = 111 bits (56), Expect = 1e-24  
Identities = 63/64 (98%), Gaps = 1/64 (1%), Frame = +1 / +1

```
Query: 1  gaacattgtgagtttggcttcatctccattgtctggaaaataatctttgagagcattgta 60
      |||
Sbjct: 633 gaacattgtgagtttggcttcatctccattgt-tggaaaataatctttgagagcattgta 691
```

```
Query: 61  cccc 64
      |||
Sbjct: 692 cccc 695
```

>[SGN-M6508](#) C2\_At1g05350-2 [cosii\_markers]

Length = 842

Score = 129 bits (65), Expect = 5e-30  
Identities = 74/77 (96%), Frame = +1 / -1

```
Query: 348 ccagatagcgtgacacctgtccgaacttcaacaagtacttcaatgtggtttgaactaaaa 407
      |||
Sbjct: 210 ccagatagcgtgacacctgtccaaacttcaacaagatattcaatgtggtttgaactaaaa 151
```

```
Query: 408 gtccagcaacaactccc 424
      |||
Sbjct: 150 gtccagctacaactccc 134
```

Score = 103 bits (52), Expect = 3e-22  
Identities = 62/64 (96%), Gaps = 1/64 (1%), Frame = +1 / -1

```
Query: 1  gaacattgtgagtttggcttcatctccattgtctggaaaataatctttgagagcattgta 60
      |||
Sbjct: 271 gaacattgtgagtttggcttcatctccattgt-tggaaaataatctttgagggcattgta 213
```

```
Query: 61 cccc 64
      |||
Sbjct: 212 cccc 209
```

## Summary

The sequencing data for primer p3-84 F2/R2 was very good. With G70 (resistant inbred) and W168 (susceptible) the sequencing data was excellent and easily comparable. When they were compared, there were absolutely no differences in over 700 base pairs. We concluded that there is no introgression in G70 at 84 cM.